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13. ABSTRACT (Maximum 200 words)

E. coli, genetically engineered with a mercury(II)-sensitive promoter and the *lux* genes from *Vibrio fischeri*, were used as microbial sensors for the detection of mercury. Evaluation of this genetic construction was carried out by determining the effects of various parameters on cell suspensions maintained at constant conditions in a small vessel. The strongest light intensities and quickest induction times occured with cells in the mid-exponential growth phase maintained at 28°C, concentrated to 1×10^9 cells/mL, mixed at very fast speeds, and aerated at 2 vvm (volume of air per volume of culture per minute) during light measurement in the small vessel. The sensitivity of these cells to the mercuric ion lied in the range of 0.02-4 µM (4-800 ppb) and the total response time was on the order of one hour, depending on the above parameters. The cells exhibited great specificity for mercury. The cells have almost equal specificity for organic and inorganic forms of the mercuric ion and responded more weakly to the mercurous ion. A simple, inexpensive, durable miniature probe was constructed and operated using the optimum parameters found in the small vessel as a guide. The range of sensitivity to the mercuric ion detected in the probe was 0.01-4 µM when aeration was provided.

14. SUBJECT TERMS

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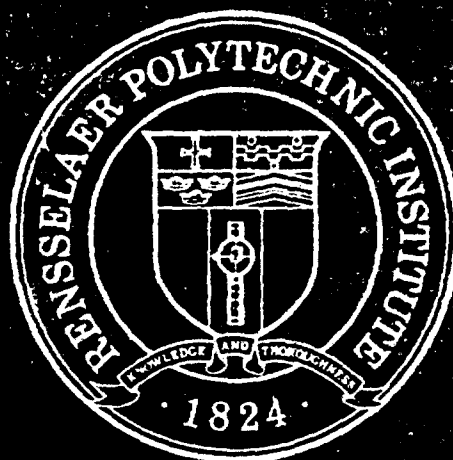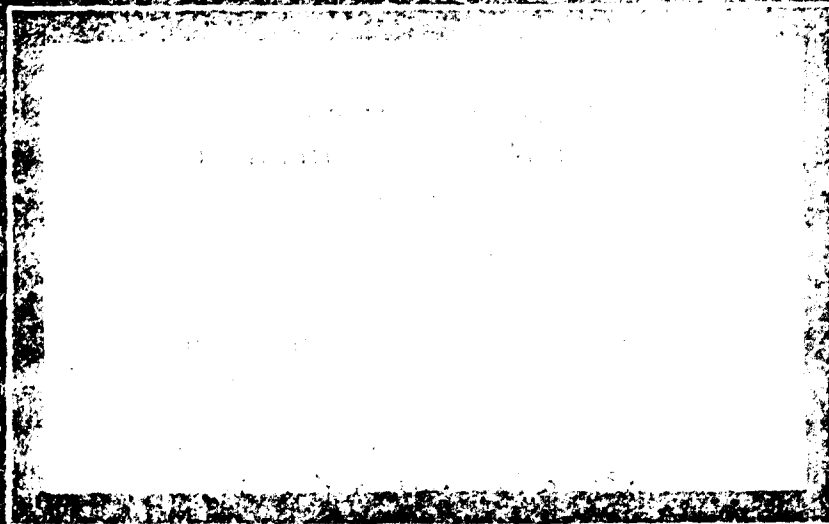
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Construction and Evaluation of a Metal Ion Biosensor

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1. OBJECTIVE.

The objective of this study was to evaluate various growth conditions on the light emitted from luminescent *Escherichia coli* and to construct instrumentation to maintain these cells viable in a practical, rugged sensor design for the detection of mercury.

2. SUMMARY.

E. coli, genetically engineered with a mercury(II)-sensitive promoter and the *lux* genes from *Vibrio fischeri*, were used as microbial sensors for the detection of mercury. Evaluation of this genetic construction was carried out by determining the effects of various parameters on cell suspensions maintained at constant conditions in a small vessel. The strongest light intensities and quickest induction times occured with cells in the mid-exponential growth phase maintained at 28°C, concentrated to 1×10^9 cells/mL, mixed at very fast speeds, and aerated at 2 vvm (volume of air per volume of culture per minute) during light measurement in the small vessel. The sensitivity of these cells to the mercuric ion lied in the range of 0.02-4 μM (4-800 ppb) and the total response time was on the order of one hour, depending on the above parameters. The cells exhibited great specificity for mercury. The cells have almost equal specificity for organic and inorganic forms of the mercuric ion and responded more weakly to the mercurous ion. A simple, inexpensive, durable miniature probe was constructed and operated using the optimum parameters found in the small vessel as a guide. The range of sensitivity to the mercuric ion detected in the probe was 0.01-4 μM when aeration was provided.

3. APPROACH.

We have focused on parameter variation in a minireactor test cell and a design for a miniature probe construction. The minireactor was a small 100-mL glass vessel that provided mixing, temperature control, and aeration to a suspended culture, and that was adapted with a fiber optic to pick up and transmit the emitted light to a detector.

Conditions of the cell suspensions could be controlled and parameters varied independently to study their effect on cell light emission. Cell suspensions were grown in culture flasks, spun down, and then resuspended and studied in the minireactor. This approach was easier than using the dual reactor test cell previously proposed that requires operation of a fermentor. The minireactor incorporated significant flexibility such that the parameters originally proposed for investigation in both the *Rose* chamber test cell and the dual reactor test cell can be studied in the minireactor alone. Optimum light intensities and induction times were found for each parameter that was varied. These optima were used as a guide in operating a 3-mL miniature probe.

4. EXPERIMENTAL PROCEDURES.

4.1 Microbial Strain

The clones were supplied by David Holmes (Clarkson University, Potsdam, NY). The *mer* operon from *Serratia marescens* and the *lux*

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genes from *Vibrio fischeri* were fused and expressed in *Escherichia coli* (strain JM109).

4.2 Cell Cultivation

For all studies, *Escherichia coli* containing the *mer-lux* plasmid were grown in a Luria broth medium (LB) with 50 μg/mL ampicillin (Sigma, St. Louis, MO). The LB medium was prepared by adding the following (in grams per liter of distilled water): bactotryptone (Difco, Detroit, MI), yeast extract (Sigma), sodium chloride (Mallinckrodt, Paris, KY), and glucose (Fisher, Fairlawn, NJ). Precultures were inoculated with a toothpick by picking a colony grown on an agar plate of the same medium. Precultures were grown overnight with shaking in 12 mL of medium. These precultures were used to inoculate culture flasks. Cultures were grown with shaking in 88 mL of medium. Except where noted, cultures were inoculated with 20% (v/v) inoculum. All culture transfers were carried out in a UV-sterilized culture enclosure (Labconco, Kansas City, MO). Cell counts were done by serial dilution and absorbance measurement at 660 nm.

Mercuric chloride (Fisher), except when varied, was used at a concentration of 0.2 μM. The other analytes were mercurous chloride, zinc(II) chloride, ferrous chloride, cupric chloride, cobaltous chloride, aluminum(III) chloride, and mercuric acetate (Aldrich, Milwaukee, WI).

4.3 Cell luminescence characterization studies

Sample Preparation. Cultures were grown for 3 1/2 hours, promptly removed, and separated into 25-mL portions for centrifugation. The cells were centrifuged at 16°C for 15 minutes at 3000 rpm. After centrifugation, one portion was used per luminescence measurement. The portions were used as needed for measurements over a 12-hour period, unless otherwise indicated. The centrifuged cells remained at the room temperature of 22°C.

Only four measurements could be made from one 100-mL culture. During the course of an experiment, however, more than four measurements were always taken, so more than one culture was needed. Generally two cultures and four precultures were used. In all the experiments, the cultures were inoculated with equivalent volumes from the four precultures and all centrifuged portions contained equivalent amounts from both cultures. This method of volume allocation or proportioning was adopted to minimize variations from measurement to measurement resulting from possible variations from culture to culture.

Light was measured in a 100-mL jacketed glass vessel (Kontes, Vineland, N.J.), the "minireactor." The centrifuged cells were resuspended and added to the minireactor. Cell counts were taken for each sample.

Light detection equipment. The minireactor was adapted with inlets and outlets for addition and removal of culture, analyte, and sterile rinse water. A glass tube was fitted into the central port

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of the minireactor for insertion of a fiber optic (Schott Fiber Optics Inc., Southbridge, MA) (core diameter = 64 mm, length = 60 cm, NA = 0.56, glass core). The effective volume of the acceptance cone that the fiber optic saw was approximately 8 mL. The minireactor was covered with an opaque cloth to remove ambient light interference. The temperature was controlled with a water jacket and maintained at 28°C, except where noted otherwise. The temperature was measured with an RTD (Omega, Stamford, CT) and recorded using a personal computer. A magnetic stir bar provided mixing. In some experiments aeration was provided through a sparger. The air was sterilized with a 0.2μ filter (Gelman Sciences, Ann Arbor, MI). The flowrate was monitored with a rotameter (Cole-Parmer, Chicago, IL). A schematic is shown in Fig. 1.

The fiber optic transmitted the luminescence to a R1527 photomultiplier tube (Hamamatsu, Bridgewater, N.J.) powered by a high voltage power supply (Pacific Instruments, Concord, CA) set at 960 volts. The electrical current from the PMT was sent to a 2A50 amplifier (Pacific Instruments, Concord, CA) with an output impedance of 50 Ω and a gain of 100, and emanated with an associated voltage. The voltage was recorded with a data-acquisition software (Unkelscope, Cambridge, MA) for one hour via a DAS-8PGA A/D board (Keithley-Metrabyte, Taunton, MA). This particular set-up gave a current (mV) to light (photons/sec) conversion of 1.25×10^5 photons/mV·sec. The efficiencies and conversion factors for the different pieces of equipment are given in Table I.

4.4 Probe miniaturization studies

Cells were grown and prepared as described above but centrifuged in three-mL quantities. Approximately 2.5 mL of a resuspended sample were added to a Union Cross fitting (Swagelok, Solon, OH) adapted as shown in Fig. 2 and the luminescence measured as described above.

A glass disk was glued into the right-hand side fitting of the cross, the "probe." The fiber optic was fitted with ferrules into this fitting. The glass disk protected it from contact with the culture. The effective volume that the fiber optic saw in the probe was approximately 1.4 mL. The whole probe was kept in a 28°C water bath. A miniature stir bar provided mixing. Aeration was provided through the left-hand side fitting; the air was filtered and its rate measured as done in the minireactor. The top fitting was cracked open for air to escape, while the bottom one was sealed tight with a Plug nut (Swagelok). The probe was light-proof.

5. RESULTS AND DISCUSSION.

5.1 Approach

Several parameters were varied in the minireactor: culture age, temperature, cell density, mercury concentration, mixing rate,

Table I. Efficiencies and conversion factors for components of the light-detection set-up.

Instrument	Parameter	Value
Fiber Optic	effective transmission (%), T	40
Photomultiplier Tube (PMT)	quantum efficiency (%), Q^a	10
	current amplification, A	4×10^5
A/D Board		50
	impedance (Ω), R	100
	gain, G	

a) Q = number of photons incident onto the PMT/ number of photoelectrons emitted from the PMT. The current (I) is calculated as follows

$$I \text{ (mV)} = \frac{P \text{ (photos/sec)} * 1.6 \times 10^{-19} \text{ (Coulomb/electron)} * A * G * R \text{ (}\Omega\text{)} * 1000 \text{ m V/V}}{Q \text{ (photons/electrons)} * T}$$

$$= 8 \times 10^{-6} \text{ (mV/photons/sec)} * P \text{ (photons/sec)},$$

where the light-to-current conversion is 8×10^{-6} mV sec photon^{-1} and its inverse, the current-to-light conversion, is 1.25×10^5 photon sec^{-1} mV^{-1}.

and aeration rate. Optimum values were found for the strength of the light emission (light intensity) and the induction time. These optima were used as a guide for operating the minisensor. A correlation with the concentration of mercury was obtained in the minisensor.

5.2 Results

5.2.1 Signal characteristics.

The cells were found to emit light approximately thirty minutes after addition of mercury. This time lag, called the induction time (I), indicates when the emitted light was *first* detected. The cells did not emit a flash of light at the induction time and then cease luminescing. Rather, the emission continued to increase for at least another 30 minutes.

Since the signal kept increasing, one had to determine when the cells will gave a sensitive correlation to the concentration of mercury. This time is called the response time (r). The total response time (R) of these microbial sensors is the summation of the induction time and response time. The light emission was not recorded for more than one hour in the minireactor because total response times of more than one hour were discarded as too long.

The signal strength in the minisensor was weaker than that in the minireactor because of the smaller volumes used, so light emission was monitored for 1 1/2 hours in order to obtain stronger light intensities. Longer total response times were required in the minisensor to obtain a sensitive correlation.

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5.2.2 Parameter variation in the minireactor test cell.

Before commencing optimization studies of the operating parameters used in the minireactor, the phase at which the cells give off maximum light needed to be found. The cells were grown for their entire growth cycle from the lag phase to the stationary phase. At different intervals 25 mL of the culture was removed, added to the minireactor, and the luminescence measured as previously described. The culture was not centrifuged prior to measurement. The growth curve of the cells for two different percent inocula is shown in Fig. 3 and the light emission during the course of the growth cycle is shown in Figs. 4a and 4b for 1% and 20% (v/v) inoculum, respectively. The cell density (Figs. 4c and 4d) and the calculated light output per cell (Figs. 4e and 4f) are also shown for 1% and 20% (v/v) inoculum. The point of maximum light emission occurs with the 20% (v/v) inoculum culture at 3 1/2 hours into the growth cycle, so for all future studies cells were taken from a 20% (v/v) inoculum culture that had grown for that length of time.

High cellular luciferase contents must exist during the early growth phases because of the high light output per cell (Figs. 4e and 4f). For the 1% (v/v) inoculum culture, a second peak occurs during stationary phase (Fig. 4b) because of the higher cell densities.

The effects of temperature on light intensity and response time are shown in Fig. 5. Maximum light intensity occurs at 26°C and minimum induction time occurs at 30°C, so 28°C was selected as a trade-off temperature for all future studies.

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The effects of cell density on light intensity and induction time are shown in Fig. 6. The light intensity increases with more cells present, but above a certain cell density, it drops off probably due to the scattering of the light by the cells.

A correlation of concentration of mercuric ion with light emission is shown in Fig. 7. The most sensitive correlation was obtained with a 30 minute response time. Longer response times were not considered, as mentioned above. These cells exhibited sensitivity for the mercuric ion in the range 4 ppb to 200 ppb (0.02-4 μM). The toxicity of mercury began to adversely effect the light emission from the cells above 20 ppb because of the absence of a complete *merA* gene. Light emission was nearly linear with concentration for one order of magnitude from 0.04 to 0.4 μM (20 ppb), where it peaked. For higher concentrations light emission was nearly linear, but inversely proportional to concentration. Bioluminescent *E. coli* which have not been cloned with a toxin-specific promoter exhibited bioluminescence quenching in the same range, 0.4-2.0 μM (1). Metabolic inhibition in *E. coli* started at a critical mercury concentration of 0.4 μM.

The mixing rate was not found to affect light intensity, although it did influence induction time. Induction time decreased linearly with mixing rate (Fig. 8). This trend indicates that the rate of diffusion of the mercury into the cells affected the response time of the cells and that faster stirring speeds could be used to shorten total response time of the microbial sensors.

Aeration increased the light intensity by 50% (Fig. 9). Thus, the luciferase-catalyzed reaction must be inherently oxygen-limited.

Above about an air flow rate of 50 ml/min, the light intensity decreased possibly due to multiple scattering. Perhaps, *E. coli* cloned with bioluminescent genes should also be transfected with genes that enhance oxygen uptake, such as the *Vitreoscella* gene constructed by Bailey et al. (2), in order for sufficient oxygen to be present for the luminescent reaction. Aeration also caused an increase in induction time, possibly due to a dilution effect from the additional air bubbles.

Sensitivity to other metal ions was tested. No light was detected from 0.2 µM of zinc(II) chloride, ferrous chloride, cupric chloride, cobaltous chloride, and aluminum(III) chloride. The mercuric ion was detected at similar light intensities and induction times for the inorganic and organic (mercuric acetate) forms. The cells were also sensitive to the less prevalent mercurous ion (Fig. 10); however, the detected signal was much weaker in both intensity and induction time.

5.2.3 Cell Pellet Age.

The long-term storage of the stock cultures used for streaking plates may have affected the luminescent behavior of cultures inoculated from these stock cultures. The cells initially gave reproducible signals for the duration of the experiments. However, after long-term storage (more than 60-75 days) of the stock cultures at -20°C and several thawing/refreezing cycles, changes in light emission were observed. The light intensity and induction time of these cells did *not* remain constant while they remained at ambient temperatures as a pellet (after centrifugation) (Figs. 11a and 11b), as they did when younger stock cultures were used. Within six hours,

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induction time droped precipitously and then leveled off. Light intensity increased with cell pellet age, the time the centrifuged cells remained at ambient temperatures, and levelled off after 8-9 hours. An interim ten-hour period, when the pellets are 10-20 hours old, existed in which light intensity and induction time did not vary significantly (Fig. 11).

The cells used for all the studies described in the previous section (except aeration rate and other metal ions) were grown from stock cultures that were less than two months old. The light intensities and induction times for those studies did not follow the trends in Figs. 11a and 11b with cell pellet age, so they are not invalidated by this effect. However, later experiments, including repetition of the variation of the aeration rate in the minireactor, test of the sensitivity to other metals, and all studies in the minisensor (see below), were carried out using cell pellets that were 10 to 20 hours old in order to minimize variation resulting from storing the pellets at ambient temperatures.

The effect of mixing rate was repeated at a later date using cell pellets that were 10-20 hours old, and it was found that mixing rate decreased linearly at nearly the same rate (slope) as before. This also indicates that the previous trends were not artifacts of cell pellet age. However, the curve shifted downward; the induction times were quicker when the stock culture used had been stored for a longer period of time (60 days longer) and undergone more freezing/thawing cycles (Fig. 8). Since cell luminescence behavior differs with storage time, the cells should be tested periodically and the probe calibrated before use.

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5.2.4 Minisensor evaluation.

Measurements in the minisensor were taken using cells with a pellet age of 10-20 hours. The mixing rate was approximately 3200 rpm. No light was detected in the minisensor without aeration (Fig. 12). An aeration rate of 15 mL/min or 6 vvm (volume of air per volume of culture per minute) was used because it gave the strongest light intensities in the minisensor. This value differs from the optimum rate found in the minireactor, 50 mL/min (2.0 vvm). The difference may be due to the fact that a sparger was used in the air inlet to the minireactor, whereas the air gently bubbled into the minisensor.

The correlation obtained with concentration of mercury in the minisensor is shown in Fig. 13. The detectable range of sensitivity to the mercuric ion is almost the same as that obtained in the minireactor, 0.01-4 μM, and parabolic in nature; although the sensitivity at the low end of the range behaved differently. The induction time was on the order of 30 minutes, as it was in the minireactor. However, a more sensitive correlation with concentration was obtained with a response time of 60 minutes than that of 30 minutes, but the total response time then was one and one-half hours instead of one hour. The peak shifted to 1.0 μM with longer response times. It must be realized, when comparing the correlations shown in Fig. 7 and Fig. 13 for the minireactor and minisensor, respectively, that there was no aeration for the correlation obtained with the minireactor, the mixing rate was much

less in the minireactor, and the effective volumes of culture that the fiber optic sees in the minireactor and minisensor are different.

6. CONCLUSIONS.

Cell density, temperature, mixing, and aeration are all important factors in maximizing light output of genetically-engineered bioluminescent *Escherichia coli*. Cells in the mid-exponential growth phase produced the strongest light intensities. Cultures in this growth phase exhibited optimum trade-off between light intensities and induction times in the minireactor when maintained at 28°C, concentrated to 1×10^9 cells/mL, mixed at very fast speeds and aerated at 2 vvm (volume of air per volume of culture per minute).

The range of sensitivity for the mercuric ion was 0.02-4 μM in the minireactor and 0.01-4 μM in the minisensor, when aerated. The correlation of light intensity and concentration of the mercuric ion in the minireactor was parabolic in nature with regions in the mid-range exhibiting linearity. The correlation obtained in the minisensor was similar to the one obtained in the minireactor.

The induction time of the cells was approximately 30 minutes. After the induction period the light intensity increases. Sensitive correlations were obtained 30 minutes beyond the induction period, resulting in a total response time of one hour.

The cells exhibited exquisite specificity for mercury, in particular for the mercuric ion. No response was detected from the other metals ions tested. Since all forms of mercury are toxic, these

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cells may prove useful in toxicity analyses and certainly require simpler protocols than those of the cold vapor methodology that are used when different forms are present.

Light emission was studied from cell suspensions. It was found that luminescence may vary with short-term durations (on the order of hours, the duration of an experiment) after long-term storage (on the order of months) of the stock cultures at -20°C. When using these cells in a probe for field testing it will be important to periodically check the cells' luminescence and to calibrate the probe prior to use. Further investigations should be carried out to test how luminescence changes over long periods. Storage at temperatures lower than -20°C may reduce variability in the light emission from the cells.

Studies need to be carried out to investigate how to maintain the cells in a static state. Hank's balanced salt solution (HBSS) has been found to stabilize the light signal from cell suspensions (1). HBSS may also enhance sensitivity because the solution is clear and less light will be scattered.

Other phases, such as lyophilization or immobilization, should be investigated for maintaining the cells in a static state. If cell suspensions cannot be maintained in a static state for a substantial period of time and will require on-site culturing, then these other phases may make eventual operation of a probe easier than cell suspensions. Variation of luminescence over time should be tested for these phases as well as the effects of storage temperatures (refrigerator, freezer, etc.) on luminescence. Åkerman et al. (3) observed changes over time in immobilized bioluminescent *E. coli*

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and they also noticed a difference with the method of storage of the beads.

7. RECOMMENDATIONS.

The trends observed for the different parameters studied can be used to improve biosensor sensitivity and response time and they can serve as a guide for probe design. Since temperature is a variable that influenced light emission, temperature control is important, but it may be difficult to obtain in the field. Providing for thermostasis with a water bath, even if small, is inconvenient.

The sensitivity of the cells and the correlation with the concentration of mercury may be further enhanced by concentrating the cells during resuspension to the optimum cell density of $1x10^9$ cells/mL. Since the correlation was parabolic, dilution may be necessary during operation in order to know which half of the correlation curve is being detected. Although, if the entire mercury-detoxifying *merA* gene could be inserted, the toxicity effects and parabolic correlation may be eliminated and the linear region extended into the range of higher concentrations.

Addition of exogenous oxygen is important because the cells are oxygen-limited. Incorporation of the *Vitreoscella* gene may enhance oxygen uptake and cell response. The effect of the addition of oxygen-carrying molecules such as hemoglobin or perfluorocarbons may also be evaluated; although, this approach may be less convenient because additional components need to be added whereas the gene would be an inherent part of the biosensor.

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In the field, if the cells are found to be oxygen-limited even with the *Vitreoscella* gene and/or oxygen-carrying molecules, air may be easily added to the probe with an inexpensive aeration pump. The effects of ambient air temperature, pressure, and humidity would have to be investigated. The *Vitreoscella* gene may also be useful if immobilized cells are used because there are even greater diffusion limitations in gel beads than in free suspension.

Since oxygen is a limiting component, limitation of other substrates may need to be tested. Addition of the other substrates, i.e., aldehyde and $FMNH_2$, may also improve cell response.

Other parameters that should be tested for their effect on cell light emission include pH, other culture media (i.e., M9 minimal medium), and the use of transparent solutions for resuspension of the centrifuged cells (minimal medium, salt solutions such as HBSS, buffers).

Greater mixing shortened cell induction time and improved diffusion of mercury into the cells. In the minireactor the sparger broke up air bubbles through convective flow producing more but smaller bubbles for increased surface area and diffusivity. It also provided additional mixing and turbulence. The minisensor, however, had no sparger. If a membrane, filter, or mesh could be inserted at the inlet of the probe, air could be forced to diffuse into the probe inducing sufficient mixing such that a stir bar may not be necessary in the minisensor. This would eliminate the need for a magnetic stirrer, increases the volume for the cells and further simplify probe operation.

The probe design may be adapted for flow-through if continuous measurements need to be made. The cells and samples tested could be compartmentalized with membranes. Although, an alternate design may be warranted.

Microbes are able to detect high levels of mercury contamination in water. The genetic construction used in this work is more sensitive than other microbial detectors. It is slightly less sensitive than cold vaporization atomic absorption spectrometry, based on values reported by the EPA, and it cannot yet compete with some newer commercially-available detectors which have even lower sensitivities (although, they do not indicate the working linear range) than those reported by the EPA.

The sensitivity of these toxin-specific microbes may be further lowered with some of the aforementioned suggestions (elimination of substrate-limitation, suspension in HBSS, concentration to higher cell density, etc.) and perhaps the parabolic correlation eliminated by altering the genetic design.

In conclusion, the studies carried out in this investigation, those proposed for future work, and the probe design presented may serve as a prototype for studying other bioluminescent cells and developing biosensors using such cells. Thus, a family of bioluminescent metal ion biosensors could be built based on the ideas presented here for the genetic design and methodology for construction and evaluation of a probe.

8. APPENDIX.

A sample measurement of a light signal recorded with the light-detection equipment described earlier is shown below (Fig. 14). Light intensities (in millivolts) and induction times were extracted from such signals. The induction time (I) is indicated. As previously defined, I is the duration for which no bioluminescence is detected and only the background level is measured. The signal coming from the photomultiplier tube is negative, so a negative deviation from the background represents light emitted from the cells. A line was drawn through the lowest values that follow the general trend of the signal; scatter data points were disregarded. Values for light intensities were calculated as the difference of the background value and the values falling on the drawn line for a selected response time.

9. PUBLICATIONS AND PATENTS.

A manuscript resulting from this research has recently been submitted for publication:

Tescione, L. and Belfort, G., "Construction and Evaluation of a Metal Ion Biosensor" submitted to Biotechnology and Bioengineering, January 1993.

No patents were filed as a result of this research.

ACKNOWLEDGEMENTS.

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LIST OF FIGURES

Figure 1. Small vessel ("minireactor") in which bioluminescence was measured.
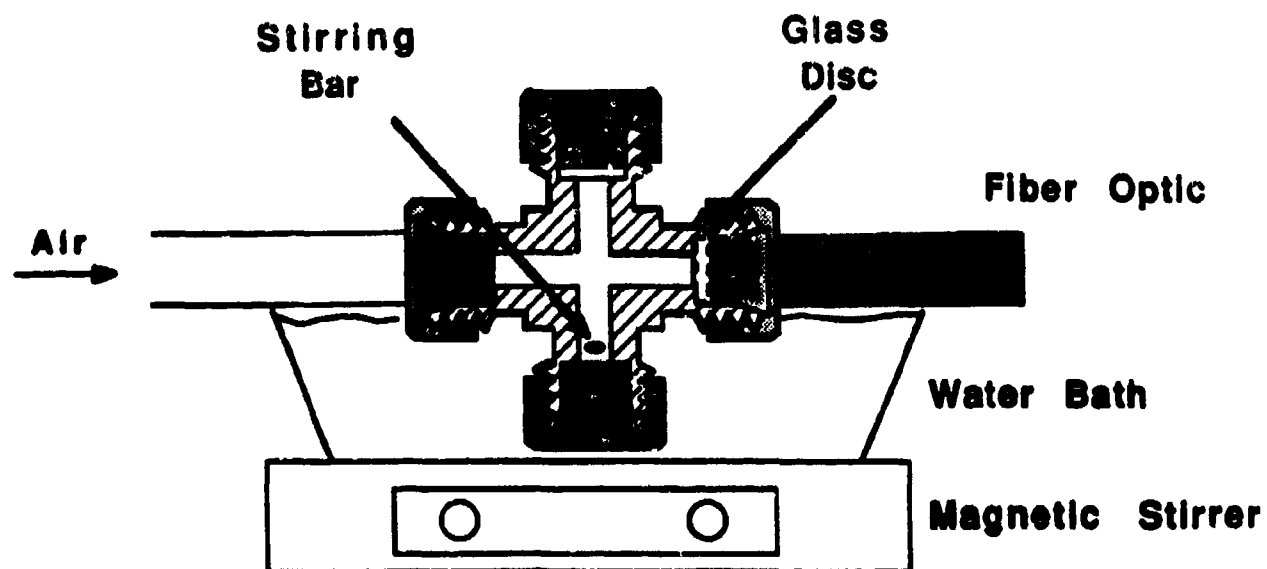
Figure 2. Miniature probe: Union Cross fitting (Swagelok, Solon, OH) adapted for aeration, mixing, and light detection. Temperature was kept constant at 28°C with a water bath.
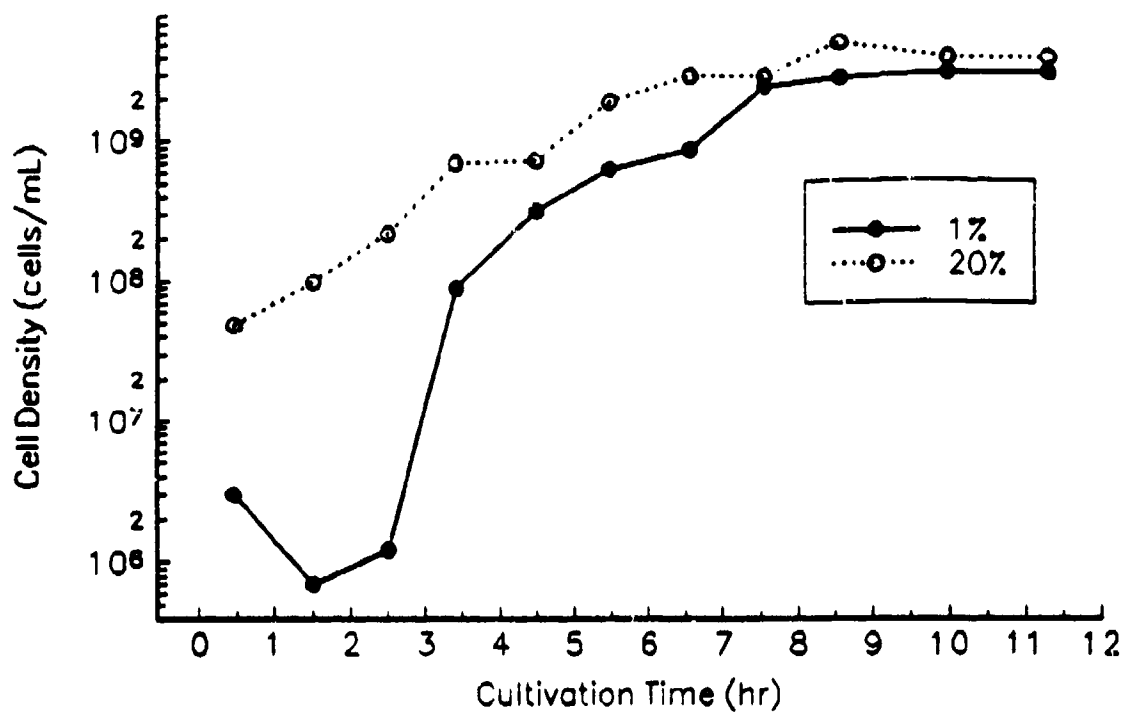
Figure 3. Growth curves for the cells when grown in a 1% and 20% (v/v) inoculum culture.
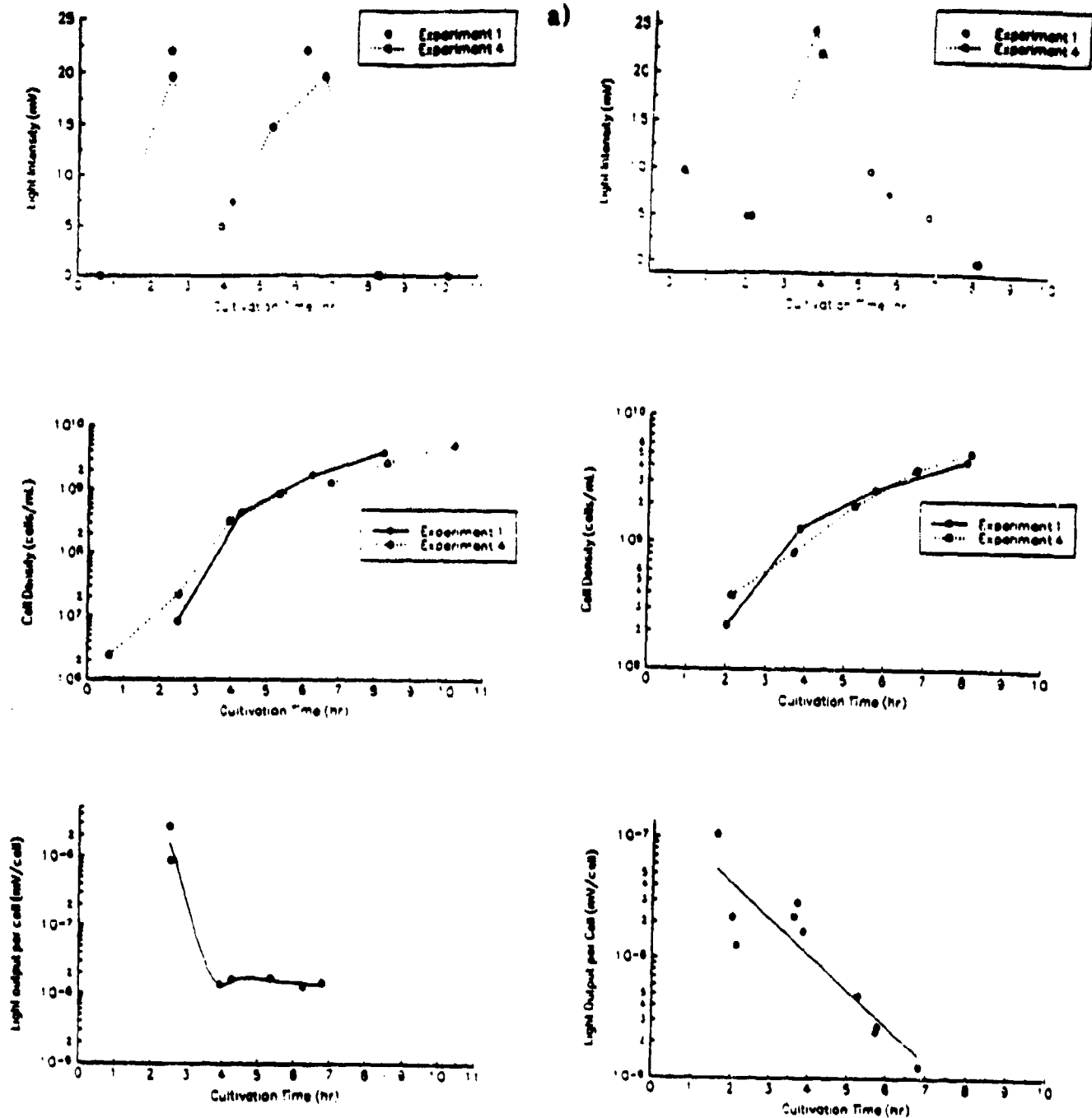
Figure 4a) Light intensity from a 1% (v/v) inoculum culture and b) a 20% (v/v) inooculum culture from two experiments; c) cell density from the 1% culture and d) 20% culture from two experiments; and e) the light output per cell for the 1% culture and f) 20% culture from the two experiments.
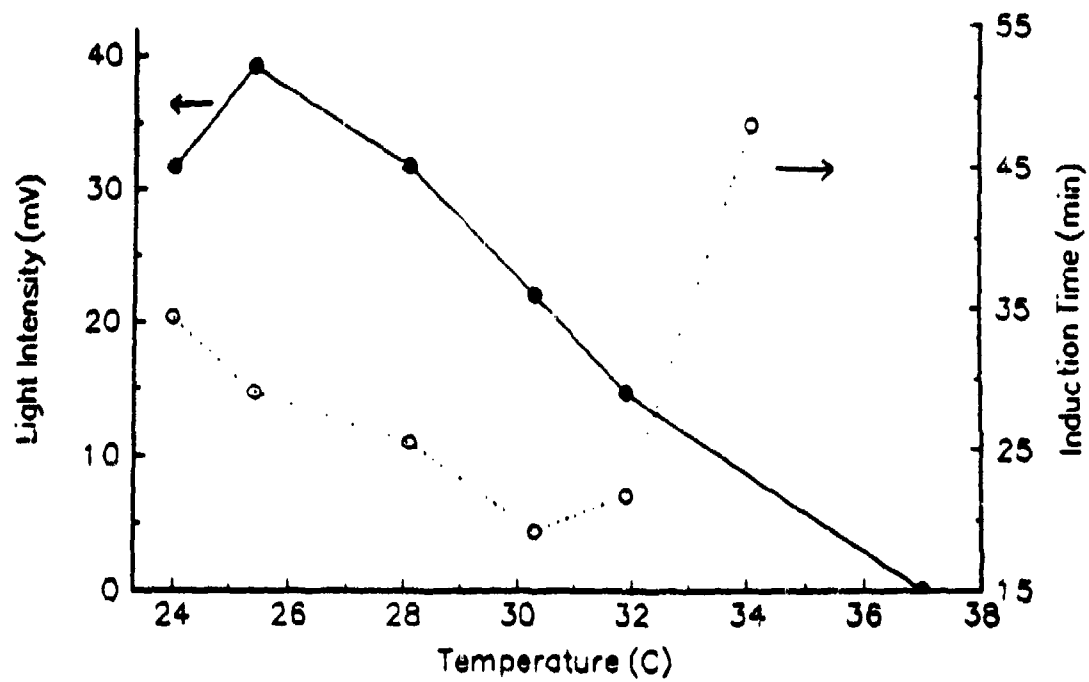
Figure 5. Effect of temperature on light intensity and induction time of the cells.
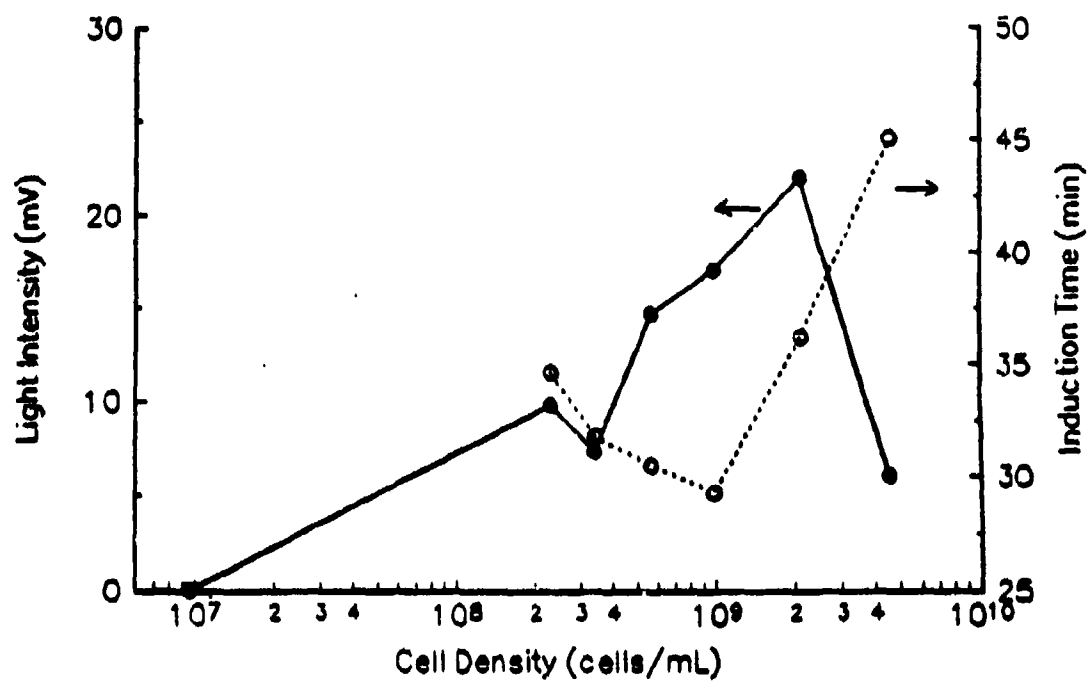
Figure 6. Effect of cell density on the light intensity and induction time of the cells.
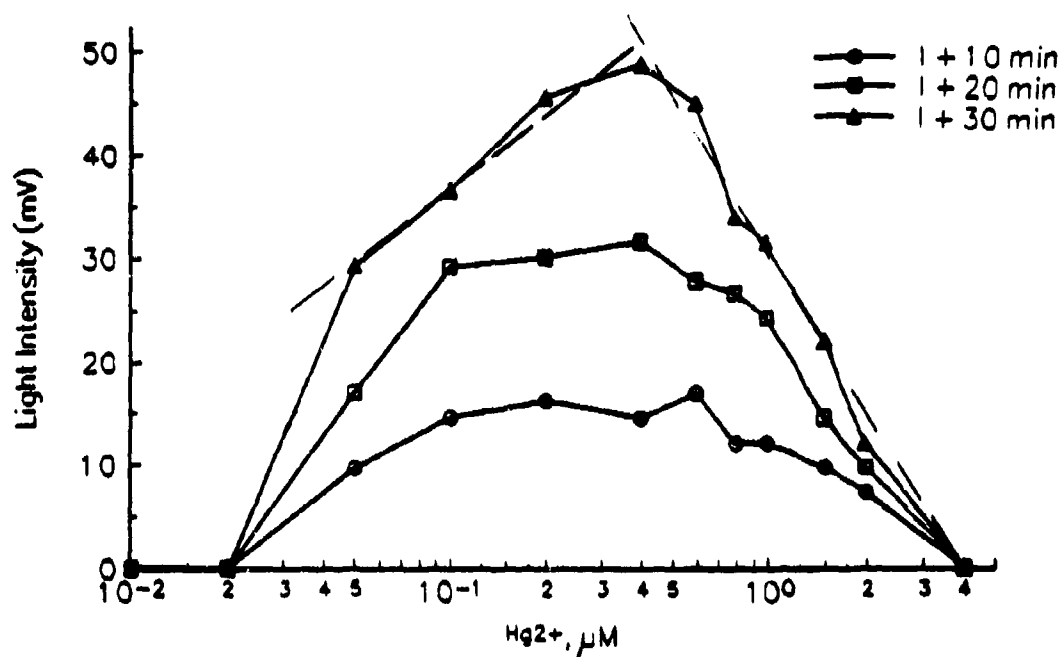
Figure 7. Correlation of light intensity with different concentrations of mercury for different total response times. Dashed lines suggest a possible linear relationship.
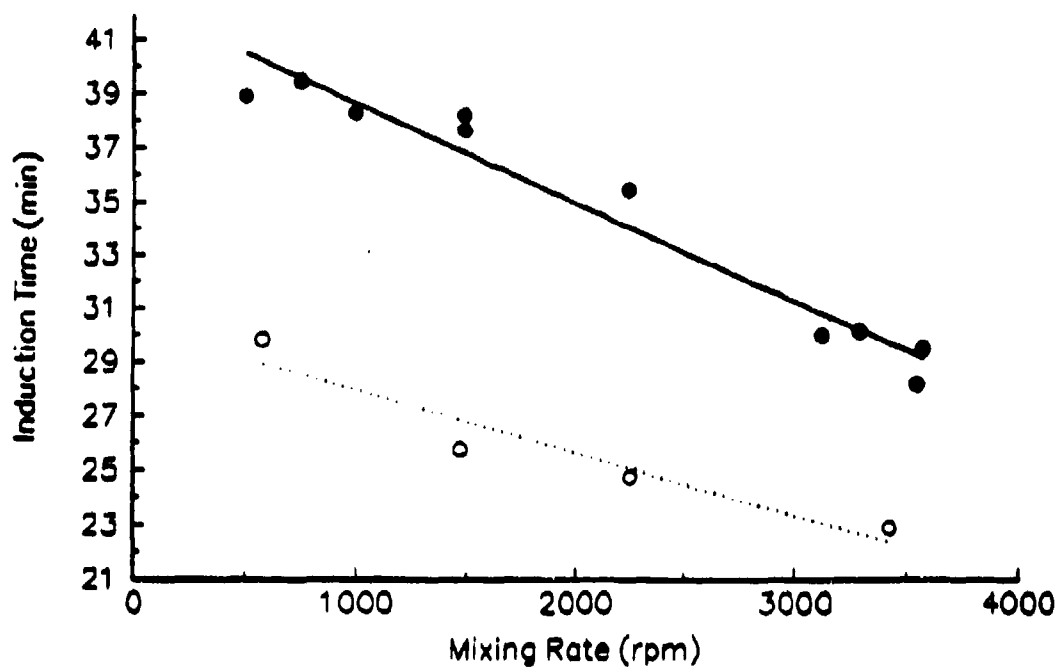
Figure 8. Effect of mixing rate on induction time using cells with a pellet age of 0-12 hours old (•) and using pellets 10-20 hours old (o).
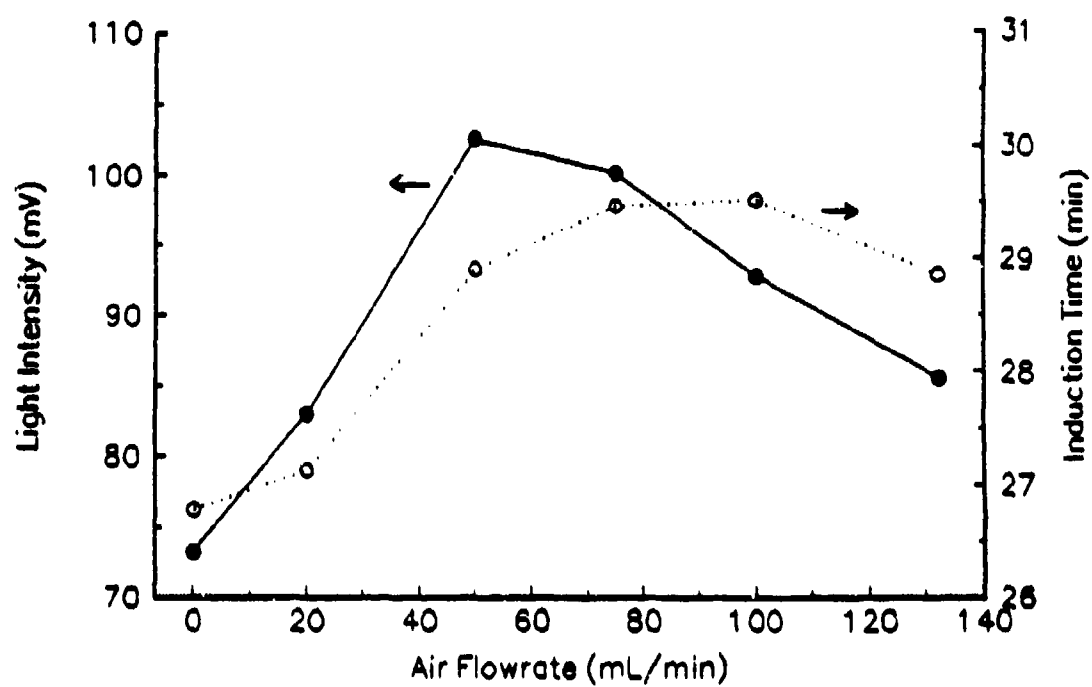
Figure 9. Effect of aeration on light intensity and induction time.
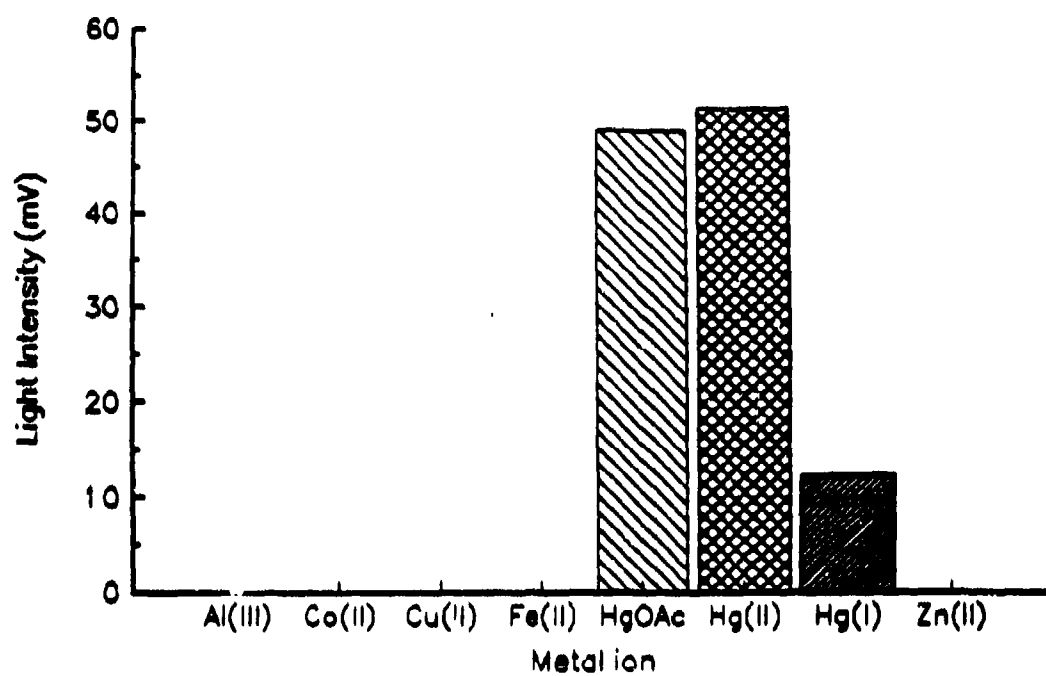
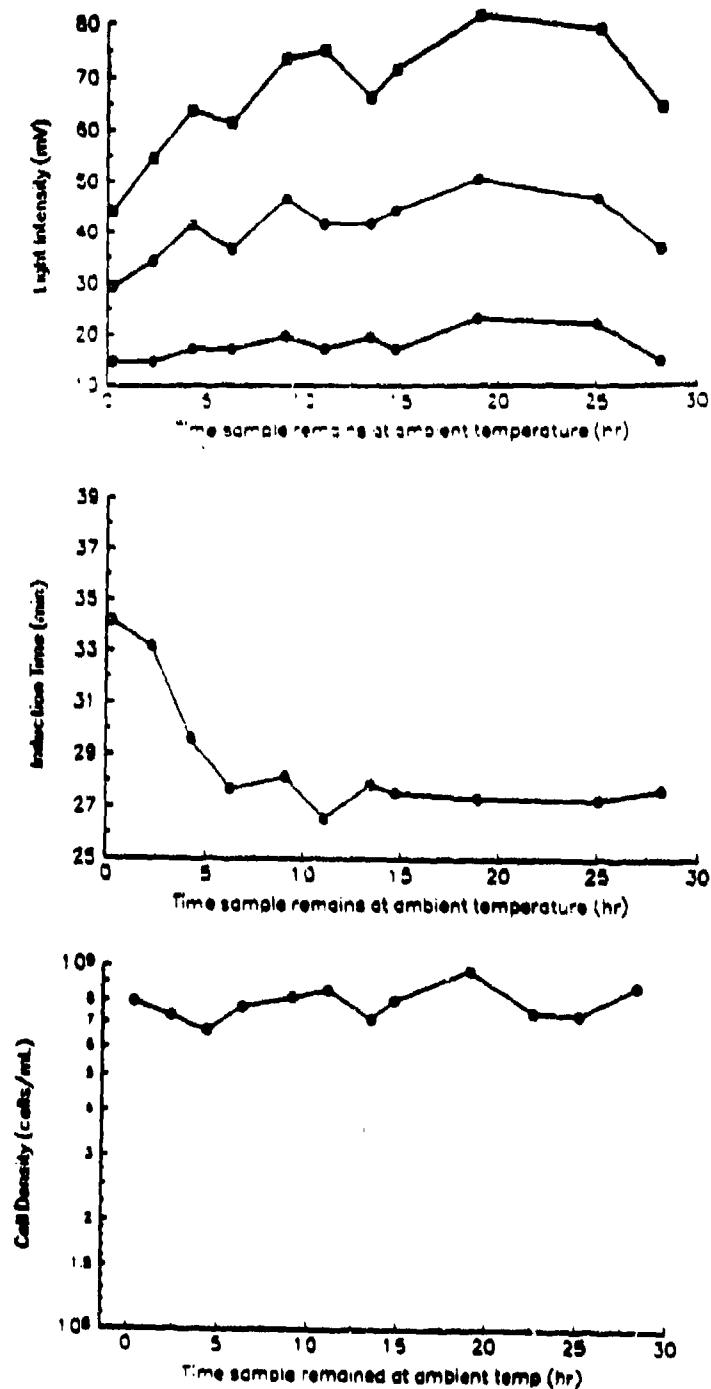Figure 10. Light intensities measured after induction with varous metals.

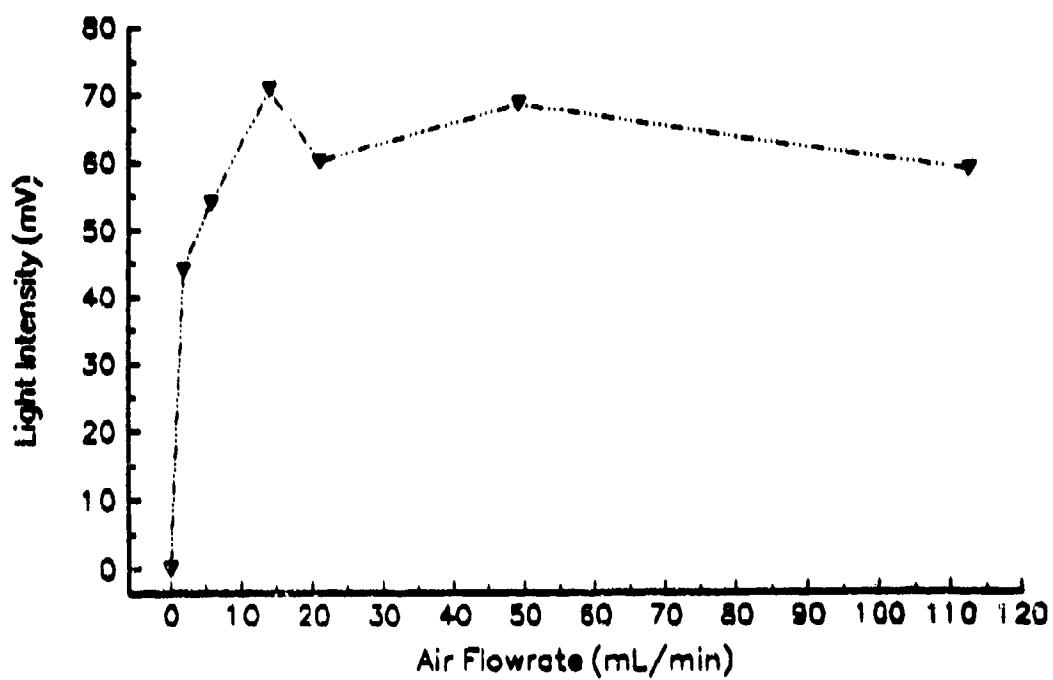Figure 11. Eff.ct of pellet age on cell bioluminescence: a) light intensity, b) induction time, and c) cell density.

Figure 12. Effect of aeration on light intensity in the minisensor (response time, r, is 60 minutes).
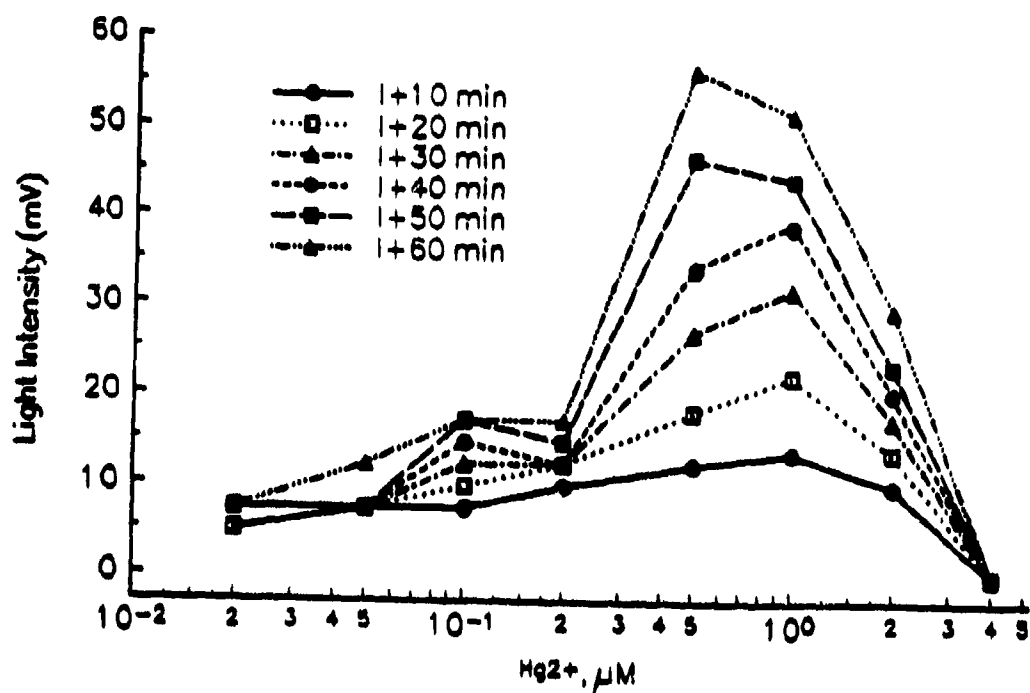
Figure 13. Correlation of light intensity in the minisensor with different concentrations of mercury for different total response times.
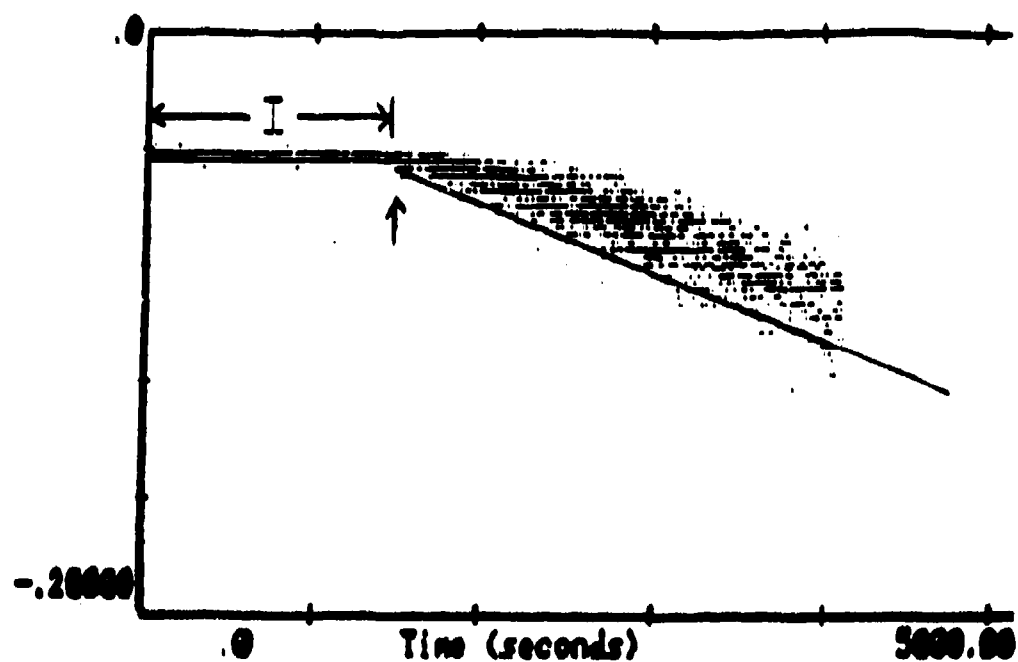
Figure 14. Sample raw data measurement and extraction of light intensity and induction time (I).

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